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Altered interleukin-2 receptor α-chain is expressed in human T-cell leukaemia virus type-I-infected T-cell lines and human peripheral blood mononuclear cells of adult T-cell leukaemia patients through an alternative splicing mechanism

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SUMMARY

A polymerase chain reaction (PCR) method was used to detect the interleukin-2 receptor α-chain (IL-2Rα) chain which lacks the conventional transmembrane (TM) domain in mRNA from human T-cell leukaemia virus type-I (HTLV-I) -infected cell lines or peripheral blood mononuclear cells (PBMC) isolated from adult T-cell leukaemia (ATL) patients. Primer pairs encompassing the TM domain were selected to generate a 357-base pair (bp) fragment. A 146-bp PCR product was observed consistently in addition to the target 357-bp PCR product in mRNA from HTLV-I-infected cell lines, such as MT-1, MT-2, MT-4 and in PBMC isolated from ATL patients. However, this 146-bp PCR product was undetectable in HTLV-I-negative cell lines. The product was also detected in PBMC from normal individuals if activated *in vitro* with phytohaemagglutinin but not without stimulation. DNA sequence analyses revealed that exons from 5 to 7, which define a 211-bp region containing the conventional TM domain, were deleted in the 146-bp PCR product. The C-terminal amino acid sequence starting from Gly^{174} of the 211-bp-deleted molecule was distinct from that of conventional IL-2Rα as a result of an altered reading frame. We identified a 45 000 MW peptide generated from IL-2Rα mRNA through this exon skip in cell lysate of MT-1 and MT-2 by Western blot analyses using an antibody raised against the peptides specific to an altered IL-2Rα. Our results indicate that an altered IL-2Rα chain is expressed in HTLV-I-infected T lymphocytic cell lines and in ATL patients.

INTRODUCTION

The interleukin-2 (IL-2) receptor (R) is unique among growth factor receptors in that it is made up of at least three distinct membrane components as a trimer chain.[1] All three chains are in close affinity with IL-2, and receptor component signalling is ensured.[2] IL-2Rα alone binds IL-2 with intermediate affinity, but does not transduce a signal. A βγ complex has intermediate affinity, capable of signalling if the IL-2 concentration is relatively high. In addition to expressing IL-2Rα, full-length protein on cell surfaces, soluble forms of IL-2Rα (sIL-2Rα) are normally present in the serum and urine of both animals and humans.[3,4] Increased levels of sIL- 2Rα have been reported in a wide variety of autoimmune, viral, parasitic and neoplastic diseases.[5–7] While most T-cell leukaemias do not express IL-2Rα, adult T-cell leukaemia (ATL) cell lines, all of which are infected with human T-cell leukaemia virus-I (HTLV-I), uniformly express large numbers of IL-2Rα.[8] Moreover, soluble forms of IL-2Rα that retain ligand-binding activity have been detected in serum of ATL patients at abnormally high concentrations.[7] In addition, the sIL-2Rα concentration appears to correlate directly with the level of progression in human immunodeficiency virus (HIV)-induced disease.[9]

In interleukin receptors, soluble forms are generated through two mechanisms: one is alternative splicing (i.e. IL-4,[10] IL-6[11,12] and IL-7[13]), with deletion of the transmembrane (TM) domain and the other is proteolytic cleavage of full-length receptors (i.e. IL-2,[14] IL-6[15]). The TM domain consisting of 19 amino acids is subject to part of exon 6 and 7 of the IL-2Rα gene.[16] To elucidate the mechanism for generation of sIL-2Rα, and to determine the existence of IL-2Rα mRNA lacking the conventional TM domain, we analysed mRNA isolated from various cell lines using a reverse transcriptase (RT)–polymerase chain reaction (PCR) method. In these studies, we demonstrated that exons 5, 6 and 7 were skipped by alternative splicing in IL-2Rα mRNA isolated from HTLV-

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Abbreviations: IL-2Rα, interleukin-2 receptor α-chain; OVA, ovalbumin; TM, transmembrane.

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I-infected cells and that the resulting molecule lacked the conventional TM domain. This report describes the existence of another type of alternative splicing in addition to that reported by Leonard *et al.*[17] in which exon 4 is simply deleted by alternative splicing. Furthermore, we show that this altered IL-2Rα generated by the exon skip is expressed in HTLV-I-infected T-cell lines. Collectively, we discuss the molecular mechanisms for the generation of the altered IL-2Rα chain from HTLV-I-infected cell lines and from peripheral blood mononuclear cells (PBMC) isolated from ATL patients.

MATERIALS AND METHODS

Cells and PBMC

Cell lines positive or negative for HTLV-I that were used are listed in Table 1. PBMC were isolated from ATL patients and healthy subjects using the conventional Ficoll–Hypaque method. The cells were maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml of penicillin, and 100 μg/ml of streptomycin. Normal PBMC (2×10^5 cells/ml) were stimulated with or without phytohaemagglutinin (PHA-P, Sigma, St Louis, MO) at the final concentration of 1 μg/ml for 72 hr.

RNA isolation, cDNA synthesis, RT-PCR amplification and cloning

Total cellular RNA was prepared using methods described by Arrigo *et al.*[18] The RNA (0·1–0·5 μg) was reverse transcribed in the presence of 2R3-1 primer for 1 hr at 37° and the resulting cDNA was amplified by the PCR.[18] The PCR was performed in a DNA thermal cycler (PC-700, ASTEC, Fukuoka, Japan) for 30 cycles of denaturation at 91° for 1 min, annealing at 62° for 1 min, followed by polymerization at 72° for 1 min. The PCR-amplified products were separated on an 8% polyacrylamide gel or a 3·5% NuSieve GTG agarose gel (FMC, BioProducts, Rockland, ME) and visualized by staining with 0·02 μg/ml of ethidium bromide (EtBr). The DNA separated on an agarose gel was extracted, then cloned into the pUC118 vector.[19] For sequence analysis of the cloned DNA, single-strand DNA was prepared and sequenced according to the standard protocols of Amersham International plc (Bucks., UK). Oligonucleotide primers used for PCR were as follows:

2R5-1, 5′CAGCTCATATGCACAGGTGA3′;

2R5-2, 5′ATGGACACCAGTCAGTTTCC3′;

2R3-1, 5′GGCAAGCACAACGGATGTCT3;

2R3-2, 5′TCCTGGGCGACCATTTAGCA3′;

Nucleotide sequencing

Nucleotide sequence analysis was performed by the standard dideoxynucleotide chain termination method[20] using commercial T7 sequencing kits (Pharmacia-LKB, Uppsala, Sweden).

Production of antiserum to synthetic peptide

Two peptides consisting of 25 and 21 amino acid residues, respectively, extending from amino acid number 177 to number 201 for SP-1 and from number 249 to number 269 for SP-2 (see Fig. 3), were generated by an altered reading frame of IL-2Rα and custom-synthesized by Seikagaku Corporation (Tokyo, Japan). Ten milligrams of each synthetic peptide was coupled to the carrier protein ovalbumin (OVA; 10 mg) in 1·5 ml of H_2O containing 30 mg of 1-ethyl-3-dimethyl-aminopropyl-carbodiimide hydrochloride at room temperature overnight.[21] Conjugates (0·5 mg/animal) emulsified in complete Freund's adjuvant (day 0) or incomplete Freund's adjuvant (day 14 and 28) were injected intramuscularly into two NZW rabbits as described previously.[22]

Table 1. Detection of PCR products for mRNA of IL-2Rα in HTLV-1-infected or non-infected cell lines and PBMC isolated from ATL patients

Cell line or patient	Cell type* (virus)	Origin or diagnosis†	PCR products‡	
			146-bp	357-bp
MT-1	T (HTLV-1)	ATL	+	+
MT-2	T (HTLV-1)	Cocultivation	+	+
467	T (HTLV-1)	ATL	+	+
HUT102	T (HTLV-1)	ATL	+	+
MT-4	T (HTLV-1)	Cocultivation	+	+
TL-0ml	T (HTLV-1)	ATL	+	+
B95-8	B (EBV)	Cocultivation	−	+
Jurkat	T	ALL	−	+
H9	T	ALL	−	+
MOLT-4	T	ALL	−	−
U937	M	LY	−	−
TAKE	PBMC	ATL	+	+
OWAK	PBMC	ATL	+	+
TIKA	PBMC	ATL	+	+
HI§	PBMC	PHA+	+	+
HI	PBMC	PHA−	−	+

* T, T cell; B, B Cell; M, macrophage; PBMC, peripheral blood mononuclear cells; EBV, Epstein–Barr virus.
† ATL, adult T-cell leukaemia; ALL, acute lymphocytic leukaemia; LY, lymphoma. PHA+, stimulated with rHA; PHA−, non-stimulated.
‡ +, positive for the PCR products; −, negative for the PCR products.
§ HI, healthy individual.

Southern blot analysis

PCR products were separated on a 3·5% NuSieve GTG agarose gel and transferred to a Hybond N membrane (Amersham).[23] For use as a probe, a 30-bp 2R4–8 primer, 5′ CTTCTACTCTTCCTCCTGGAAACTGACTGG, was synthesized based on the combined sequence of the 3′-terminal 15 bases of exon 4 continuous with the 5′-terminal 15 bases of exon 8, respectively. The 2R4-8 primer was labelled with [γ-^{32}P]ATP and hybridization carried out at 60° for 72 hr in a QuikHybri buffer (Stratagene Cloning System, La Jolla, CA).

Western blot analysis

Samples of 5×10^6 cells were washed twice with phosphate-buffered saline (PBS). The cells were then treated for 20 min at 4° with 0·5 ml lysis buffer [10 mM Tris–HCl, pH 8·0, 0·1 M NaCl, 3 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulphonylfluoride, 0·5% nonidet P-40 (NP-40)]. Lysates were cleared by centrifugation at 15000 *g* for 10 min at 4°. Proteins were run on 10% sodium dodecyl sulphate (SDS)–polyacrylamide gels. After transfer to nitrocellulose sheets (Clear blot membrane-P, AE6660, Atto Corporation, Tokyo, Japan), blots were treated overnight with blocking buffer [20 mM Tris–HCl, pH 7·6, 150 mM NaCl, 0·2% Tween-20 (TBS-T), and 2% FCS]. After washing with TBS-T, samples were incubated with rabbit polyclonal antibodies (anti-SP-1 or anti-SP-2) diluted 1: 500 with TBS-T for 1 hr at 18°. After further washing with TBS-T, blots were treated with anti-rabbit immunoglobulin G–horseradish peroxidase (Cappel, Durham, NC) as appropriate, and tagged proteins were detected by enhanced chemiluminescence (ECL, Amersham).

RESULTS

Detection of alternatively spliced IL-2Rα mRNA lacking the transmembrane domain-coding region

We investigated whether the IL-2Rα molecule lacking the TM domain was detectable in the mRNA of various cell lines. The mRNA from cultured cells was isolated and cDNA synthesized using the primer 2R3-1. Next, these samples were subjected to PCR. A region which flanks the TM domain was amplified using the oligonucleotide primer pair 2R5-1 and 2R3-1, then nested PCR was carried out using primers 2R5-2 and 2R3-2, to generate a 357-bp fragment (Fig. 1). As shown in Fig. 2(a), all samples produced comparable amounts of the expected 357-bp fragment plus a smaller size (146-bp) fragment (lanes 1–6) except for B95-8 and Jurkat, which exhibited the apparent absence of the smaller fragment (lanes 7 and 8). It is probable that the DNA of the 357-bp fragment was directed by the conventional cDNA of IL-2Rα. On the other hand, the DNA of the smaller-sized fragment might have been produced through the deletion of a considerable region of IL-2Rα mRNA by some mechanism, such as alternative splicing or an exon-skip (see below). Similar analyses were conducted for several other cell lines and are summarized in Table 1. The smaller products were detected consistently in HTLV-I-positive cell lines, such as MT-1, MT-2, 467, MT-4, TL-Oml. In contrast, the 146-bp fragment was not expressed in B95-8, and Jurkat cell lines, although the conventional 357-bp fragment was expressed equally. The HTLV-I-negative cells, such as B95-8, Jurkat and H9 did not produce 146 bp fragments, although these cells produced the 357 bp fragments (Table 1).

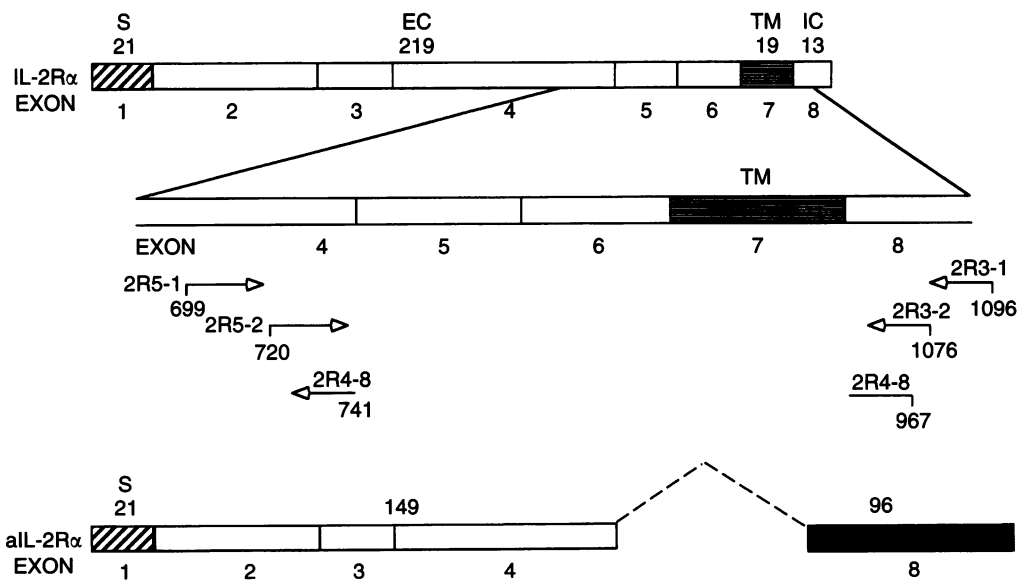

Figure 1. Structures of IL-2Rα mRNA and the alternative splicing product. The portion of IL-2Rα mRNA with the location and orientation of the oligonucleotide primers marked by arrows, used for PCR is schematically depicted in the centre. Altered IL-2Rα is depicted by aIL-2Rα. Numbers at the arrows show positions of the nucleotides according to the report by Leonard *et al.*[17] The oligonucleotide 2R4-8 is composed of 3′ portion of exon 4 juxtaposed to 5′ part of exon 8. S represents the signal sequence; EC, the extracellular domain; TM, the transmembrane domain; and IC, the intracytoplasmic domain. Numbers above the mRNA indicate residues of amino acids represented by these domains. The solid box in aIL-2Rα indicates that the amino acid sequence is distinct from that in the conventional IL-2Rα, as a result of an altered reading frame. Broken lines indicate regions lacking aIL-2Rα mRNA.

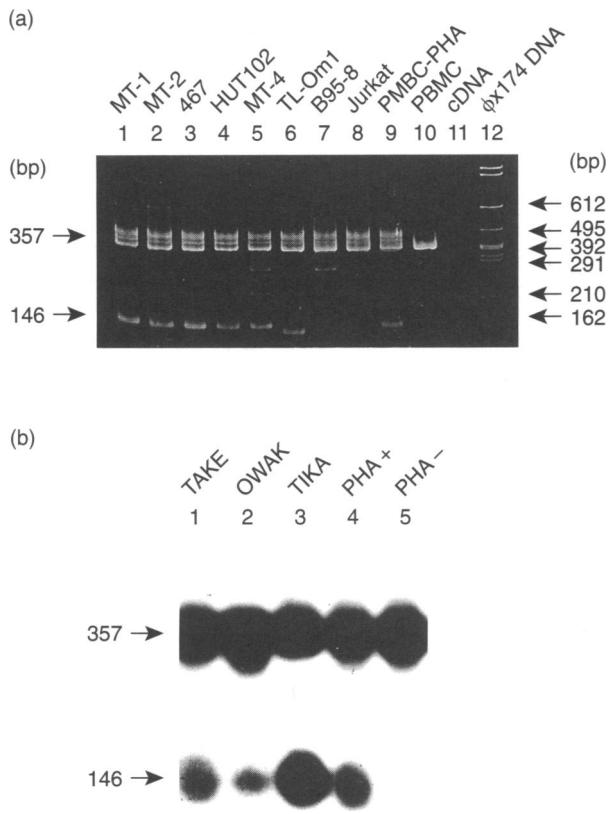

Figure 2. Detection of alternatively spliced mRNA for the IL-2Rα in various cell lines. RT-PCR analyses were carried out as described in the Materials and Methods. Portions (10 μl) of each PCR product were analysed on a 10% polyacrylamide gel. The DNA was visualized by ultraviolet fluorescence after staining with ethidium bromide (EtBr). (a) Lane 1, MT-1; lane 2, MT-2; lane 3, 467; lane 4, HUT102; lane 5, MT-4; lane 6, TL-Om1; lane 7, B95–8; lane 8, Jurkat; lanes 9 and 10, PBMC isolated from healthy individuals; lane 9, stimulated with PHA; lane 10, non-stimulated; lane 11, without template cDNA; lane 12, ØX174 DNA digested by *Hin*dIII restriction endonuclease. (b) Detection of the alternatively spliced mRNA for IL-2Rα in PBMC of ATL patients or a healthy person. The PCR products (10 μl) were separated on a 3·5% NuSieve GTG agarose gel, transferred to a nitrocellulose sheet, and hybridized with 5′-end-labelled 2R4-8 primer (see Fig. 1). Lanes 1, 2 and 3, ATL patients; lanes 4 and 5, healthy individual; lane 1, TAKE; lane 2, OWAK; lane 3, TIKA; lane 4, stimulated with PHA; lane 5, non-stimulated.

The TL-Om1 cells produced a slightly smaller-sized PCR product than the 146-bp fragment in addition to the 357-bp PCR product, suggesting that additional regions were deleted from the mRNA of IL-2Rα (Fig. 2a, lane 6).

Since IL-2Rα mRNA from HTLV-I-positive cell lines contained an unconventional form, similar experiments were performed on samples from ATL patients. PBMC were obtained from ATL patients or healthy individuals and the RNAs were isolated. The samples were subjected to RT-PCR analyses as described above, and the products were separated on an agarose gel. Detection of IL-2Rα DNA was carried out by Southern hybridization using 5′-end-labelled 2R4-8 oligonucleotide, which detects both conventional and alternative IL-2Rα. The 146-bp fragment in addition to the 357-bp fragment was again detected in PBMC from all the ATL patients examined (Fig. 2b). Larger size DNA bands than 357 bp or smaller size DNA bands than 146 bp were weakly visible. However, these faint DNA bands were not shown in Fig. 2(b) because these were derived from the 2R4-8 oligonucleotide, which has a 13 nucleotide long sequence complementary to 2R5-2 primer, and might hybridize to non-specific PCR products carrying 2R5-2 primer. Main DNA bands, the 357-bp and 146-bp fragments were confirmed to be derived from IL-2Rα mRNA by sequencing analysis (data not shown). Moreover, both fragments were evident in the PBMC from healthy individuals when stimulated with PHA (Fig. 2b, lane 4), however, the 146-bp fragment was not observed without stimulation (Fig. 2b, lane 5). The results of PCR analyses for mRNA of IL-2Rα isolated from ATL patients are shown in Table 1. The 146-bp PCR products were detected in the mRNA of PBMC from all of the ATL patients and activated PBMC of healthy individual.

Nucleotide sequence of PCR products

In order to delimit the deleted region of the altered IL-2Rα mRNA, both DNA fragments in the samples from MT-1, MT-2 and PBMC obtained from the ATL patient, TIKA, were cloned into plasmid pUC118 and sequenced (data not shown). The smaller DNAs were uniform irrespective of their origins and showed the same deletion spanning the region from exon 5 to exon 7 (Fig. 3). Since the TM domain resides at this deleted region, it is obvious that the smaller PCR product is devoid of this conventional TM domain. In contrast, the DNA sequence of the larger PCR product (357-bp) completely matched that reported by Leonard *et al.*[17] and neither a deletion–addition nor a point mutation was detected in samples from the individuals tested, at least in this particular region (data not shown). Consequently, the amino acid sequence of this molecule lacking the 211-bp nucleotide was different from that of the conventional IL-2Rα in the region extending from amino acids no. 174 to no. 269. The C-terminus of this variant molecule included 96 new amino acids because of a frame-shift. The predicted amino acid sequence is underlined in Fig. 3. These findings were confirmed by two additional independent RT-PCR assays followed by DNA sequencing. The mRNA lacking the 211-base product was also confirmed by cloning-sequencing in the other cell lines, 467 or MT-4, and PBMC of ATL patients (TAKE) (Table 1).

Detection of altered IL-2Rα protein

The altered IL-2Rα mRNA was thus present in a particular set of cell lines. Since this mRNA is predicted to code a chimeric protein with an IL-2Rα peptide at the N-terminal two-thirds and a 96 amino acid stretch, totally unrelated to the conventional IL-2Rα at the C-terminus, we attempted to identify such a protein molecule in cells expressing an IL-2Rα mRNA. To identify the altered IL-2Rα, an antibody which specifically reacts with the altered IL-2Rα was raised in rabbits using peptides included exclusively in the new C-terminal stretch (see Fig. 3 and the Materials and Methods). The cell lysates from MT-1, MT-2, Jurkat and MOLT-4, were analysed by Western blot analysis using antisera against SP-1 or SP-2. As shown in Fig. 4, the antibody against the synthetic peptide SP-2, reacted with a 45000 MW polypeptide in MT-1 and MT-2 cells that expressed an altered IL-2Pα mRNA (lanes 1

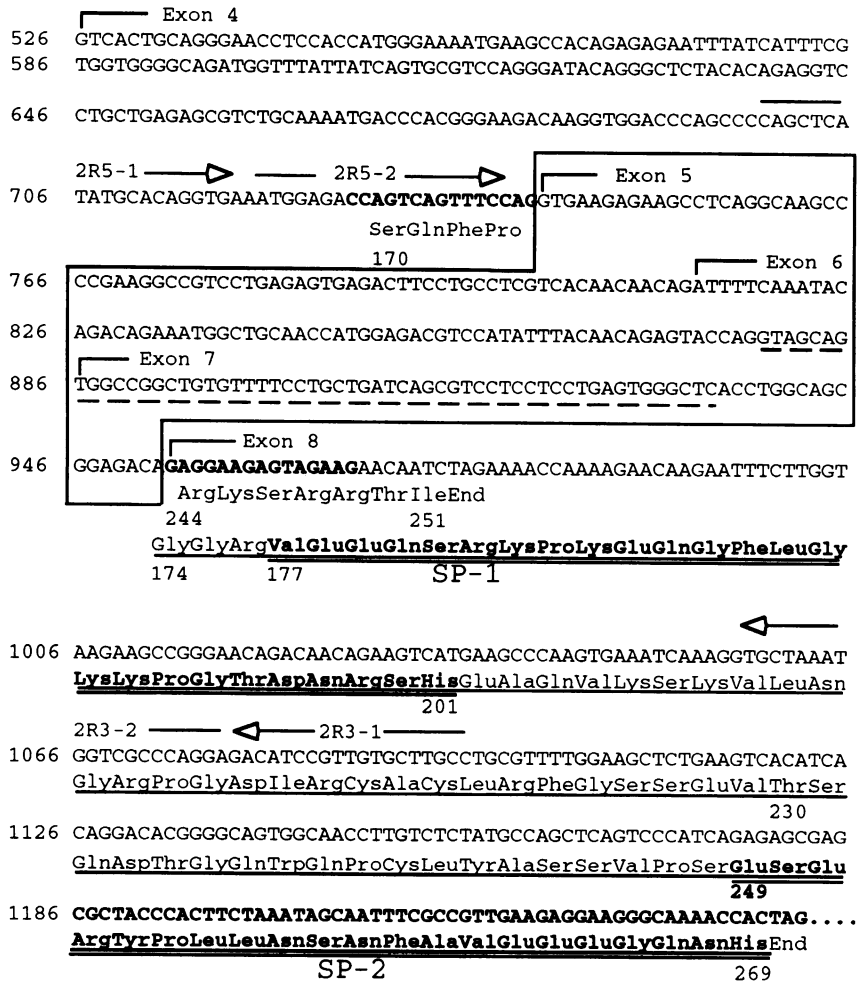

Figure 3. Nucleotide sequence and putative amino acid sequence of aIL-2Rα generated by alternatively spliced mRNA isolated from MT-1 cells. The aIL-2Rα is lacking the region shown by the box, which includes the entire TM domain (broken underlined). Nucleotide sequence analysis was performed for the region between primers 2R5-2 and 2R3-2. The sequences for other regions are quoted from the report described by Leonard *et al.*[17] The amino acids following no. 174 are distinct from those in the conventional IL-2Rα as a result of an altered reading frame. The presumed 96 new amino acids, deduced from the nucleotide sequence are underlined. Numbers at the left show positions of nucleotides. Numbers below letters refer to amino acid positions. Bold letters of nucleotides show the region of primer 2R4-8, with the skip from exon 4 to exon 8. Bold lettering of amino acids from Val^{177} to His^{201} and Glu^{249} to His^{269} show regions of synthetic peptides, SP-1 and SP-2, respectively, and double-underlined.

and 2). This antibody was however, unreactive against Jurkat and MOLT-4 cells in which no altered IL-2Rα expression was apparent (lanes 3 and 4), indicating the absence of an altered IL-2Rα protein. Production of the 45 000 MW protein was confirmed by additional independent Western blot analyses using MT-4 and HUT102 cells (data not shown). PHA stimulated PBMC was not examined. OVA was employed as a carrier protein for the synthetic peptide SP-1 or SP-2 during immunization of the rabbits. In addition to the 45 000 MW protein, several bands were commonly detected in all cell lysate samples. These may have arisen from reactions with anti-OVA antibodies because similar bands were seen in lanes 5 and 6 in which anti-OVA antibody was applied. The 45 000 MW signal was, however, barely detectable in the culture supernatants of MT-1 or MT-2 (data, not shown).

The 45 000 MW protein was detected in the cellular protein of MT-1 or MT-2 cells using anti-SP-2 antibody, but was undetectable when anti-SP-1 antibody was used. This may have resulted from poor antigenicity of the SP-1 region or steric hindrance between the mature protein molecules.

DISCUSSION

It has been reported that certain HTLV-I-positive cell lines spontaneously release large quantities of IL-2Rα into culture supernatants.[3] To determine the mechanism of generation of sIL-2Rα, we checked for the presence of a deletion of the conventional TM domain in mRNA of HTLV-I-infected cells by an RT-PCR method. Two PCR products, 357-bp and 146-bp fragments, were detected consistently in HTLV-I-positive cell lines. In contrast, the smaller-sized PCR product (146-bp) was not detected in HTLV-I-negative cell lines, whereas the larger product was expressed equally. To obtain a more detailed understanding of the generation of this 146-bp PCR product, PCR-amplified DNA was cloned in a plasmid. Cloning-sequencing confirmed the skip of exons 5, 6 and 7,

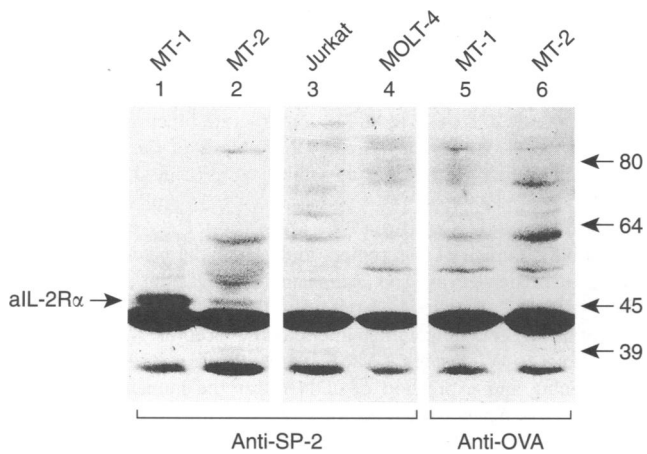

Figure 4. Detection of altered IL-2Rα peptides by Western blot analysis. The cellular proteins from MT-1, MT-2, Jurkat and MOLT-4 cells were separated on a 10% SDS–polyacrylamide gel, transferred to a nitrocellulose membrane, and reacted with an antibody specific to altered IL-2Rα, anti-SP-2. Lanes 1 and 5, MT-1 cells; lanes 2 and 6, MT-2 cells; lane 3, Jurkat; lane 4, MOLT–4. The antisera used for blotting were anti-SP-2 (lanes 1–4) and anti-OVA (lanes 5, 6).

and the direct joining of exon 4 and 8 in IL-2Rα mRNA. The same result was obtained by the use of PBMC isolated from ATL patients. Moreover, this phenomenon was also observed in PBMC from healthy individuals when activated by PHA, but absent without activation. These observations indicate that this particular type of exon-skip in IL-2Ra may be associated with HTLV-I infection or T lymphocyte activation (see below).

We prepared two synthetic peptides, SP-1 and SP-2, to raise antisera for Western blot analysis of the newly generated C-terminal amino acid stretch. In the search for altered IL-2Rα protein, a peptide with a molecular weight of 45 000 was discovered the cell lysate of MT-1 and MT-2 cells. In contrast, this 45 000 MW protein was not detected in Jurkat and MOLT-4 cells. These results indicate that the altered IL-2Rα peptide is indeed expressed in HTLV-I-infected cells.

Since the TM domain of the conventional molecule is encoded mainly by exon 7 (Fig. 3), it was clear that the altered IL-2Rα does not possess the original TM domain. However, due to the possible presence of a similar functional domain in this new C-terminal 96 amino acid stretch region, a hydrophobicity analysis[24] of this sector was conducted, revealing a hydrophobic portion from amino acid no. 232 to no. 248 (data not shown). However, since the relevant stretch length of this potential TM region is rather short with weak hydrophobicity value, altered IL-2Rα probably does not participate in membrane anchoring. An anti-SP-2 antibody specifically reacted with only the intracellular C-terminal region of IL-2Rα, but not the extracellular region. To determine whether the altered IL-2Rα molecules might be anchored in the cell membrane, immunofluorescence assays were carried out using anti-SP-2 antibody. HTLV-I-positive cell lines MT-1 and MT-2, and HTLV-I-negative cell lines MOLT-4 and Jurkat were stained. All of these cells were, however, stained equally well irrespective of their HTLV-I positivity (not shown). This was probably because of non-specific staining by anti-OVA antibody contained in the anti-SP-2 antibody preparation as mentioned above.

Since information about the existence of these molecules in culture supernatant is crucial for the elucidation of the mechanism of generation for sIL-2Rα, we attempted to determine the location of these molecules by Western blot analysis using anti-SP-2 antibody. However, we failed to find altered forms of IL-2Rα, because detection of altered IL-2Rα was probably hampered by the presence of other material, such as antibodies from FCS in the culture supernatants. As a result, various polypeptides with the same size as altered IL-2Rα were observed in cell culture supernatants and even in fresh RPMI-1640 medium. Perhaps, antibodies present in the FCS component, may bind to protein A–sepharose.

The quantity of mRNA usually reflects the product amount, and thereby would help to evaluate the physiological significance. Accordingly, we sought to measure the relative quantities of altered mRNA to normal mRNA by Northern blotting assay. However, clear separation by agarose gel electrophoresis was not achieved since these mRNA do not differ significantly in terms of molecular size. In a previous study by Robb *et al.*,[14] analyses of the sequence of soluble IL-2Rα molecules (42 000 MW) obtained from culture supernatants, showed that a soluble form of IL-2Rα is produced by proteolytic cleavage. In this study, we were unsuccessful in attempts to show that altered forms of IL-2Rα contribute to the accumulation of soluble IL-2Rα in culture media. The release of exon-skip IL-2Rα protein into cell culture supernatant requires further clarification.

Many viral or higher eukaryotic pre-mRNAs are spliced differentially so that multiple proteins are generated from a single gene with increased coding capacity from overlapping reading frames.[25] Alternative splice site selection has been shown to be mediated by proteins that are not integral constituents of the general splicing machinery. These proteins act in either a positive or negative manner to determine alternative splice site selection.[25,26] HTLV-I is known to express splicing-related proteins, such as *Tax* and *Rex,* which may play a role in the viral life cycle as well as in cellular functions. Indeed, it is known that a $p40^{tax}$ of HTLV-I regulatory protein activates viral gene transcription, and has also been found to bind indirectly to the NF-κB site of cellular chromosomes. Thus, $p40^{tax}$ activates expression of the cellular genes for IL-2 and IL-2Rα in T-cell lines by enhancing promoter activity.[27,28] Virtually nothing is known as yet about the physiological roles of HTLV-I in the enhancement of alternative splice site selection. However, the enhancement of alternative splicing is not restricted to HTLV-I-infected T cells, but is observed also in normal PBMC when activated by PHA. Derangements in splicing may not therefore be generally applicable to many cellular genes, but may rather operate in a limited number of genes, including IL-2Rα. The observations that, even in IL-2Rα, the splice alteration appeared to be concentrated around exon 4 also support this notion. Genomic sequences around exon 4 and/or intron 4 (and possibly around exon 8) are the most likely targets of regulatory proteins that may be induced or activated by HTLV-I infection or growth stimulation. The mechanisms of IL-2Rα exon skipping in HTLV-I-infected cells requires further inspection.

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